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We have focused on measuring the removal of ultraviolet (UV) light induced cyclobutane pyrimidine dimers (CPD) from the dyhydrofolate reductase gene (DHFR) in wild type and BRCA2 defective human cell lines to determine if BRCA2 gene defects impact transcription-coupled repair (TCR) in human cells. We have focused on characterizing TCR defects in human cells since other laboratories have focused on and documented TCR defects in mouse embryo fibroblast cell lines containing BRCA gene defects (Gowan et al,1998). To extend these important findings we have investigated whether BRCA2 defects impact TCR in human cells by studying the Capan-1 cell line obtained from the American Type Culture Collection. Capan-1 contains a mutation in one allele of the BRCA2 gene and a deletion in the other allele (Abbott et al, 1998). We have optimized conditions to measure TCR in human cells using a probe for the DHFR gene. We find that the Capan-1 cell line undergoes what appears to be a form of cellular senescence and interpretation of repair results are complicated by the senescence phenotype. Hence, we have chosen to establish systems where we can introduce mutant and wild type copies of BRCA genes using an inducible expression system.

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Isabel Keller 2.19.99

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Introduction

BRCA1 gene defects have been associated with defects in transcription-coupled repair of oxidative damage (Gowen et al, 1998; Abbott et al, 1999) and BRCA2 gene defects have been associated with hypersensitivity to ionizing radiation and a reduced capacity to process double-strand breaks (Abbott et al, 1998). Since BRCA1 and BRCA2 gene defects are clearly associated with a large percentage of hereditary breast cancers, these findings support roles for deficiencies in the ability to carry out repair in the etiology of breast cancer. In addition, since transcription-coupled repair is dependent on transcription, these findings may reflect a role of BRCA1 in the transcription process. We originally proposed to study the role of transcription-coupled repair in breast cancer and proposed studies to measure repair and transcription in breast tumor cell lines and BRCA gene defective cell lines. In light of the more recent findings cited above we have focused our initial studies on the studies we proposed in the original application on the role of BRCA genes in transcription-couple repair. In addition, since work published by another group soon after funding of this application has focused on repair in rodent systems and oxidative damage (Gowen et al, 1998), we have chosen to focus on our work described in the original application using human cell lines and UV damage.

Body

1. Repair of UV damage in established human cell lines:
One of our original tasks was to measure the removal of UV damage in human tumor cell lines and BRCA gene mutant mouse embryo fibroblast cell lines. Since another group published repair studies using BRCA1 mutant mouse embryo fibroblasts (Gowen et al, 1998) at the time our work was initially funded and they are also focused on BRCA 2 mouse cell lines, we decided to focus our attention on our proposed experiments to study human cell lines. To perform these experiments required the use of human strand-specific RNA probes for the DHFR gene. Use of this probe was highly problematic in that under conditions established by other investigators it lacked reproducibility and often times lacked sufficient sensitivity for the repair assay. While one previous member of the laboratory had some success with this probe, other members of the laboratory had very limited success. To remedy this problem, conditions for using this probe were optimized and were found to require several modifications of the procedure which included alterations in the hybridization buffer and temperature and methods for in vitro transcription used in generating radio-labeled RNA strand-specific probes. The optimization of these procedures has resulted in reproducible results and sufficient signal to quantify repair in several human cell lines using either X-ray film or a phosphoimager.

We have initiated our repair experiments by studying the tumor cell line, Capan – 1. We chose this cell line because it has been shown to have defects in both alleles of BRCA2. Several attempts at growing sufficient quantities of cells required for the repair assay (5-10 x 10^7 cells) were problematic in that the cells appeared to contain a growth defect and undergo some sort of cellular senescence. Since our ability to optimize the human DHFR probe has been successful we are attempting to repeat these experiments using smaller numbers of cells since optimization of the probe has resulted in a significantly greater sensitivity which requires less cellular DNA for the repair assay. However, since we and several others have identified a growth defect in BRCA mutant cell lines we are establishing other approaches to measure the consequences of mutations in these genes on repair of UV induced DNA damage. In addition we are now examining repair in human breast tumor cell lines with no known defect in BRCA1 or BRCA2 which was also one of our initial aims.

2. Construction of mutant human cell lines:
Working with established cell lines is often complicated by the presence of mutations in a variety of genes throughout the genome that have occurred during selection of the cell line or growth of the original tumor that the cell line was derived from. Hence we are constructing cell lines to obtain a better system to examine the consequences of mutations in BRCA 1 and 2 on transcription-coupled repair in human cells. We have used the ecdysone expression vector system (InVitrogen) to engineer expression constructs containing repair genes under the control of an insect hormone responsive element. We have established control experiments using the human XPA gene which is required for the removal of UV-induced cyclobutane pyrimidine dimers from either strand of an active gene and from the genome overall. We have introduced these constructs into a human XPA mutant cell line and measured expression of the protein, UV survival and DNA repair. Using confocal microscopy and western analysis we have determined that this approach results in the ability to control regulation of the gene using the insect hormone ponasterone. Induction of the gene results in resistance to UV irradiation and restoration of transcription-coupled repair of UV damage. We are currently using this system to construct BRCA1 mutant alleles, introduce them and overexpress them in wild type cells to measure their effects on transcription-coupled repair of UV damage. This approach should help alleviate the growth defects of some of the mutant alleles since we can grow cells in the absence of induction and induce expression of the gene just prior to UV irradiation.

Key Research Accomplishments

- Established conditions to measure repair in the human DHFR gene.
- Investigated repair of UV damage in the Capan-1 human cell line and find that due to growth defects in this cell line it is not optimal for these studies.

- Established a system to express wild type and mutant gene constructs using the ecdysone expression system (InVitrogen) and have used this system to correct defective DNA repair of UV damage in a mutant cell line.

Reportable Outcomes

None

Conclusions

Defects in BRCA genes are associated with defects in repair in rodent and human systems. Given the strong association between these gene defects and hereditary forms of breast cancer it should be extremely important to understand the role of defects in DNA repair in the etiology of breast cancer. However, there are significant differences in BRCA1 gene homology between rodents and humans and large differences in repair capabilities between rodents and humans. Hence, it is important to determine how mutations in these genes impact DNA repair in humans. In addition while BRCA gene defects in sporadic breast tumors is uncommon, defects in DNA repair may also be present in the sporadic tumors as a consequence of other gene defects. We and others have found cellular growth defects associated with BRCA gene mutations and the use of some of the established cell lines appears to have some limitations. Hence, we are constructing cell lines with inducible genes to overcome some of these limitations and pursuing the investigation of transcription-coupled repair in sporadic tumor cell lines.

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